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The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements

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"Capsule": *Glomalin may be useful in remediation of toxic elements in soils.*

Abstract

Naturally occurring soil organic compounds stabilize potentially toxic elements (PTEs) such as Cu, Cd, Pb, and Mn. The hypothesis of this work was that an insoluble glycoprotein, glomalin, produced in copious amounts on hyphae of arbuscular mycorrhizal fungi (AMF) sequesters PTEs. Glomalin can be extracted from laboratory cultures of AMF and from soils. Three different experiments were conducted. Experiment 1 showed that glomalin extracted from two polluted soils contained 1.6–4.3 mg Cu, 0.02–0.08 mg Cd, and 0.62–1.12 mg Pb/g glomalin. Experiment 2 showed that glomalin from hyphae of an isolate of *Gigaspora rosea* sequestered up to 28 mg Cu/g in vitro. Experiment 3 tested in vivo differences in Cu sequestration by Cu-tolerant and non-tolerant isolates of *Glomus mosseae* colonizing sorghum. Plants were fed with nutrient solution containing 0.5, 10 or 20 μM of Cu. Although no differences between isolates were detected, mean values for the 20 μM Cu level were 1.6, 0.4, and 0.3 mg Cu/g for glomalin extracted from hyphae, from sand after removal of hyphae and from hyphae attached to roots, respectively. Glomalin should be considered for biostabilization leading to remediation of polluted soils.
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Keywords: Potentially toxic elements; Metal-stabilization; Bioremediation; Fungal cell wall; Soil organic matter

1. Introduction

1.1. Organic compounds sequester high levels of potentially toxic elements (PTEs)

The terms heavy metals, toxic metals or trace elements are applied to a large group of elements, but these terms are not completely satisfactory. A more convenient term for these elements, potentially toxic elements (PTEs), has been suggested (Gadd, 1993; Alloway, 1995).

It is know that fungal cell-wall components, which contain free amino, hydroxyl, carboxyl and other groups, can be excellent binding sites for Cu^{2+} ions in fungi and plants (Zhou, 1999; Kappor and Virarghavan, 1995). Proteins, because of their well-established interaction with Cu^{2+} and other metals, are potentially one of the main cell components for binding elements outside of the cell walls (Ross, 1994; Gardea-Torresdey et al., 1997). Filamentous soil fungi such as: *Aspergillus niger*, *Mucor rouxxi*, *Rhizopus arrhizus*, and *Trichoderma viridae* sorb Cu between 2 and 10 mg Cu/g dry mycelium. These fungi are used as commercial biosorbants of PTEs (Morley and Gadd, 1995; Kapoor and Virarghavan, 1995; Mullen et al., 1992). Similar PTE-binding processes have been proposed for ectomycorrhizal and ericoid mycorrhizal fungi (Denny and Wilkins, 1987; Bradley et al., 1982), and AMF appear to have similar processes (González-Chávez et al., 2002).

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These authors showed that AMF have the ability to sorb Cu. They reported values of 3–14 mg Cu/g dry AMF hyphae. Although these authors did not discuss the nature of this sorption, we propose that glomalin is involved.

Macaskie and Dean (1990) emphasized that a variety of secreted microbial products play an important role in sequestration of large amounts of PTEs at the cell wall level. The interaction of metals with proteins is well known (Spiro, 1981) and proteins also can sequester metals. For example, a glycoprotein (hydroxypedine) from the cell wall of *Chlamydomonas reinhardtii* (Cain and Allen, 1980) and a polymer from *Zoogloea ramigera* (Sterritt and Lester, 1986) are capable of sequestering significant amounts of Cd.

There is evidence that arbuscular mycorrhizal fungi (AMF) are able to withstand potentially toxic elements PTEs (González-Chávez et al., 2002). Few studies to clarify the ecological role of AMF in PTE-polluted soils.

1.2. Glomalin

Glomalin is a glycoprotein copiously produced by all AMF tested to date (Wright et al., 1996, 1998; Nichols, 2003). Because AMF colonize 80% of vascular plant species (Trappe, 1987) and are found worldwide in almost every soil, glomalin has been detected in numerous soils in large amounts (Wright and Upadhyaya, 1998; Nichols, 2003). The structure of glomalin has not been completely defined. The molecule appears to be a complex of a repeated monomeric structures bound together by hydrophobic interactions (Nichols, 2003) that attaches to soil to help stabilize aggregates. The molecule contains tightly bound iron (0.04–8.8%) (Rillig et al., 2001; Nichols, 2003; Wright and Upadhyaya, 1998), and does not contain phenolic compounds such as tannins (Rillig et al., 2001). Preliminary evidence (Wright and Upadhyaya, 1998; Nichols, 2003) suggests that cations are bound to glomalin in amounts that vary for different soils.

1.3. Objectives

The objectives of this study were to determine whether PTEs could be detected on glomalin from polluted soils and then to examine the possible interactions of PTEs with glomalin in controlled experiments using Cu as the proxy ion. Three separate experiments were performed. Experiment 1 examined PTE concentration in glomalin extracted from polluted soils. Experiment 2 tested the efficacy of glomalin produced by an isolate of *Gigaspora rosea* to sequester or bind Cu in vitro. Experiment 3 examined the efficacy of glomalin produced by two different isolates of *Glomus mosseae* to sequester or bind Cu in vivo.

2. Materials and methods

2.1. Experiment 1: Glomalin PTE-sequestration in polluted soil

Two polluted soils that showed differences in PTE concentrations were chosen (Table 1). These soils were sampled from Devon Consol Mines, England. Sites are located 1.6 km north of Gunnislake at an elevation of 90–120 m above sea level (González-Chávez et al., 2002). Four replicates of 1-g samples were processed to extract glomalin by the following procedure: 8 ml of 50 mM citrate, pH 8.0, was added to the samples and they were autoclaved for 1 h (Wright and Upadhyaya, 1996). After centrifugation, the supernatant was removed and a second extraction was performed. Supernatants were combined for each replicate sample. In the crude extracts Bradford total proteins (BTP) and immunoreactive glomalin (IRG) were determined according to Wright and Upadhyaya (1998). Glomalin was precipitated by slowly adding 3 N HCl until the pH of the solution reached 2.5. The precipitate was re-dissolved in 100 mM sodium borate, pH 9, dialyzed against water and freeze-dried. Dried glomalin was weighed and then digested with HNO_3 and analyzed for PTE content by atomic absorption spectroscopy (AAS) following Bradford et al. (1975) methodology using a Perkin Elmer 3110 (Norwalk, CT, USA) instrument.

2.2. Experiment 2: In vitro Cu-sequestration by glomalin from an isolate of Gigaspora rosea

Gigaspora rosea (FL224) was grown on for 3 months on maize (*Zea mays*) in sand-based pot cultures supplied with low phosphorus nutrient solution (Millner and Kitt, 1992). Glomalin has been previously extracted from hyphae with 50 mM sodium citrate (pH 8.0), precipitated at pH 2.5 with HCl, re-solubilized in 100 mM sodium borate (pH 9.0), dialyzed against water, and freeze dried. A five mM copper ($CuSO_4$) aqueous solution, pH 4.5 was added to 5 mg/ml glomalin in 100 M

Table 1
Total and DTPA-bioavailable potentially toxic elements (PTEs) from two polluted soils

	Cu	Zn	Cd	Pb	Fe	Mn
Total PTEs (mg/kg soil)[a]						
Soil 1	355	Trace	4	1487	1603	268
Soil 2	1045	963	4	1250	1606	251
DTPA-Available PTEs (mg/kg soil)[b]						
Soil 1	27.6	3.40	0.20	15.0	16.0	26.4
Soil 2	35.4	2.10	0.03	0.86	26.0	7.90

[a] Determined by International Standard Organization (ISO)-method BS7755 (British Standard, 1995).

[b] 50 mM DTPA, 10 mM TEA, and 10 mM $CaCl_2$ (Lindsay and Norvel, 1978).

MES (2-[*N*-Morpholino]ethanesulfonic acid) pH 4.5 for a final volume of 500 μl. The control was glomalin without the addition of Cu. Glomalin precipitated when the two solutions were mixed. The precipitate was re-solubilized using four different procedures to determine possible Cu desorption from the glomalin by citrate, borate and hydrochloric acid (Table 2). All dialyzed samples were freeze-dried. Copper concentration in the supernatants was measured by AAS. Freeze-dried glomalin was weighed and digested with concentrated HNO_3 for 3 h and heating at 80 °C and Cu content was determined by AAS as described above. Each treatment consisted of four replicates, except the control with eight replicates. Differences among treatments were tested by ANOVA followed by Tukey's test ($P = 0.05$).

2.3. Experiment 3. In vivo Cu-sequestration by glomalin using two isolates of Glomus mosseae

Glomus mosseae BEG-25 (fungus isolated from non-polluted soils) and *Gl. mosseae* BEG-132 (from As and Cu polluted soils) were tested for their capacity to sequester Cu under greenhouse conditions. Coarse sand was used as the substrate in 15 cm diameter pots (total volume 1300 cm^3). The sand was acid-washed with 3 N HCl and then glomalin was extracted with 50 mM citrate (pH 8.0 for 1 h at 121 °C). *Sorghum vulgare* var. KS 524 was used as the host plant. Seeds were sterilized with 10% commercial sodium hypochlorite. Inoculum and seeds were placed together in the center of the pot in a bag made of 38-μm nylon mesh to form a root compartment and a root-free area for extraradical hyphae and sand. Plants were watered automatically three times a day with a standard nutrient solution (Millner and Kitt, 1992) containing 0.5 μM Cu and 0.5 mM MES to maintain the pH at 6.1 ± 0.2. High P (40 μM) nutrient solution was used for the first week for all plants and then low P (20 μM) nutrient solution (Millner and Kitt, 1992) was used for the remainder of the experiment. At the third week after planting, the nutrient solution was amended with two levels of $CuSO_4$ (10 and 20 μM) using 0.5 mM MES to maintain the pH at 6.1 ± 0.2 for the test treatments until plants were harvested. Controls received the basic nutrient solution that contained 0.5 μM Cu.

Plants were grown for a total of 9 weeks (6 weeks of exposure to different Cu levels). At harvest sand outside of the mesh bag was submerged in water and stirred vigorously to release hyphae. Hyphae were collected by decanting the water over a series of stacked sieves (250, 150, and 53 μm). The sand was washed and the washings were decanted three times. Hyphae were trapped on the 150- and 53-μm sieves and sand was trapped on the 250-μm sieve. Glomalin was extracted from hyphae using 100 mM borate (pH 9.0) at 121 °C in sequential 1-h extractions until the supernatant was colorless. Glomalin also was extracted from the sand remaining after hyphae were removed and roots from the mesh bag using 50 mM citrate (pH 8.0) at 121 °C in 1-h sequential extractions until the supernatant was pale brown. Glomalin was purified by precipitation at pH 2.5, re-dissolved in 100 mM borate, pH 9.0, dialyzed against water and freeze-dried. Cu content of glomalin was determined by AAS after digestion in concentrated nitric acid. The experiment was a complete factorial of two inoculation treatments by three Cu levels by six replicates in randomized blocks design. Differences among Cu-levels for sources of glomalin were tested by ANOVA factorial analysis including as factors: Cu levels and fungi. When differences were found, Tukey's test ($P = 0.05$) was performed.

3. Results and discussion

3.1. Experiment 1—Glomalin PTE-sequestration in polluted soil

Glomalin appears to be efficient in sequestering different heavy metals, especially Cu, Pb and Cd found in high concentrations in two polluted soils (Table 3). Differences between the two soils in sequestration of heavy metals by glomalin are probably not because a mixture of proteins with different sequestration properties is co-extracted with glomalin. Conditions required to solubilize glomalin are harsh. Other glycoproteins that we have tested do not survive the long exposure to 121 °C required to release glomalin from soil or hyphae.

Table 2
Normal and modified procedures for glomalin extraction, precipitation and purification

Treatment	Extraction at 121 °C for 1 h	Precipitation	Final Treatment(s)[b]
1 (Normal)[a]	50 mM sodium citrate pH 8.0	Hydrochloric acid	Re-solubilized in 100 mM sodium borate, pH 9, and dialyzed against water
2	50 mM sodium citrate pH 8.0	None	Dialyzed against water
3	100 mM sodium borate pH 9.0	Hydrochloric acid	Re-solubilized in 100 mM sodium borate, pH 9, and dialyzed against water
4	100 mM sodium borate pH 9.0	None	Dialyzed against water

[a] Procedure described by Wright and Upadhyaya (1996).
[b] After dialysis samples were freeze-dried.

Table 3
Bradford total proteins (BTP, mg protein/g soil), immunoreactive glomalin (IRG, mg/g soil), immunoreactivity percentage (%IR), and potentially toxic elements (mg/g glomalin) content that has been extracted and purified from two polluted soils

Soil	BTP	IRG	%IR	Cu	Zn	Cd	Pb	Fe	Mn
1	1.19 (0.12)	0.67 (0.006)	57.2 (6.5)	1.55 (0.05)	1.57 (0.62)	0.02 (0.01)	0.62 (0.06)	18.8 (3.23)	1.88 (0.15)
2	0.49 (0.04)	0.18 (0.01)	37.6 (5.8)	4.29 (0.30)	1.70 (0.11)	0.08 (0.02)	1.12 (0.16)	44.7 (4.93)	1.88 (0.28)

Assay values are means for four replicates. Standard deviation is presented in parentheses.

In previous studies glomalin extracted from pot-cultured hyphae and from soils shows the same banding pattern on sodium dodecyl sulfate polyacrylamide gel electrophoresis (Wright and Upadhyaya, 1996) indicating that large amounts of extraneous proteins are not present. Nuclear magnetic resonance (^{1}H NMR) spectra for hyphal and soil glomalin indicate that only proteins with almost the same structure (i.e. same amino acids and complexity) could possibly be present (Nichols, 2003). Glomalin for NMR spectra were subjected to the normal processing described in Table 2 for glomalin from the polluted soils examined in this study.

Soil 1 glomalin concentration was greater than that of Soil 2 as measured by Bradford total proteins (BTP), immunoreactive glomalin (IRG) and percent immunoreactive BTP (IR%). Immunoreactivity indicates a molecular configuration similar to the configuration of glomalin on hyphae (Wright et al., 1996; Wright, 2000). However, glomalin from Soil 1, which contained higher concentrations of PTEs (Table 1), sequestered lower amounts of PTEs. Overall, these results are interesting due to the high toxicity of elements sequestered by glomalin.

3.2. Experiment 2—In vitro Cu-sequestration by glomalin from Gigaspora rosea

Glomalin from *Gi. rosea* (FL224) removed Cu from solution. Glomalin precipitated immediately when 5 mM Cu and 5 mg/ml glomalin were mixed. The supernatant contained an average of only 0.34% Cu (0.19–0.50%), indicating that Cu was precipitated on glomalin. At lower Cu concentrations (1–4 mM) precipitation was noticeably slower than at 5 mM. No precipitation was observed with 0.5 mM Cu. Glomalin without the addition of Cu did not give readable measurements of Cu. The Cu content in glomalin was statistically different ($P > 0.0001$) for the four different procedures used to solubilize the Cu–glomalin pellet (Tables 2 and 4). The greatest amount of Cu sequestered by glomalin after these treatments was 28 mg/g glomalin which represented 35% of the total Cu added. The most remarkable observation was that Cu was not released totally by treatments such as citrate and hydrochloric acid. This suggested that Cu was sequestered by glomalin, not only by electrostatic Cu-sorption, but also by strong complex formation.

Differences in Cu content in glomalin after different re-solubilization treatments showed that citrate and hydrochloric acid, and possibly dialysis against water desorbed Cu bound to the molecule. When citrate was used to re-solubilize the Cu–glomalin pellet followed by precipitation with HCl, 65–93% of the added Cu was desorbed. This suggested, in part, that the chemical nature of the reaction between glomalin and Cu involves weak association as Cu released from glomalin occurred during precipitation in HCl. In this case, ion exchange may be the principal mechanism for Cu sequestration. On the other hand, it also was possible that a strong and irreversible sequestration occurred for at least 7.2–12% of the added Cu (Table 4). This suggested that at least some glomalin–Cu complexes are more stable than chloride–Cu and citrate–Cu complexes, because even after citrate extraction, HCl precipitation and sodium borate neutralization, some Cu was still present in glomalin. Unlike the chloride–Cu, the citrate–Cu equilibrium constant is high ($\log\beta_{1\ [ML]} = 18$), indicating that glomalin–Cu affinity may be stronger than predicted. Microbial products, such as siderophores from *Pseudomonas putida* (Chen et al., 1994), have a strong affinity for Cu^{+2} ions ($\log\beta_{1\ [ML]} = 22.3$).

Cu sequestration by purified glomalin was higher than Cu-sequestration by glomalin extracted from the polluted soils. Nevertheless, it is important consider that glomalin also may sequester other elements besides Cu such as Pb, Cd, Mn, Zn, and Fe. Iron content of glomalin was examined because crude and purified glomalin has a red-brown color when extracted from hyphae or soils (Wright and Upadhyaya, 1998; Rillig et al., 2001;

Table 4
Copper content in glomalin reconstituted by four different procedures from a pellet precipitated by the addition of 5 mM copper to 5 mg glomalin

Treatment sequence[a]	Copper (mg/g glomalin)	Retained Copper%
1. Citrate, HCl, borate	11 (0.94) b	12
2. Citrate	26 (7.73) a	20
3. Borate, HCl, borate	6.7 (2.47) b	7.2
4. Borate	28 (1.05) a	35

Assay values are means for four replicates. Standard deviation is presented in parenthesis. Different letters indicate significant differences for comparison made among treatments according to Tukey's test ($p = 0.05$). LSD = 8.6

[a] See Table 2 for details.

Nichols, 2003). However, other cations may also be on glomalin. It is probable that extraction and purification procedures for glomalin from soil also release other cations that may occupy binding sites in the molecule such as Ca, Mg, and K. Release of cations upon exposure to HCl also is supported by previous work showing desorption of these kinds of cations from the hyphae of AMF after a 5 min exposure to 1% HCl (González-Chávez et al., 2002). Therefore, purified glomalin may have greater potential sites to bind PTEs than glomalin in soils.

Purification of microbial products apparently can increase the capacity to sequester PTEs. For example, a polymer isolated from a freshwater-sediment bacterium was able to bind up to 2.20 mg of Cu/g of crude cell-free exopolymer preparation. The highly purified exopolysaccaride preparation bound up to 14.8 mg Cu/g of carbohydrate. Similar Cu-binding capacity was obtained for an exopolysaccharide produced by *Xanthomonas campestris* (Mittleman and Greesey, 1985). Extraction and purification procedures should be optimized to avoid underestimating the capacity of glomalin to sequester metals.

A preliminary test for Cu sequestration using sources of Cu-containing ligands other than SO_4^{-2} was preformed. Glomalin–Cu precipitate formed in all Cu sources tested: $Cu(NO3)_2$, $CuCl_2$ and $Cu(CH_3COO)_2$ (data not shown). Therefore, glomalin precipitated Cu independently of the Cu ligand. It is known that SO_4^{-2} forms complexes with some substances and stronger complexes with Cu than NO_3^-, Cl^- and $CH3COO^-$ do, but our preliminary tests ruled out Cu precipitation by $[Cu\ SO]^o$, $[Cu\ (SO_4)_2]^{2-}$ or $[Cu\ (SO_4)_3]^{4-}$ (Ringbom, 1963) or glomalin–SO_4 instead of by Cu–glomalin. Slow precipitation of glomalin was observed with Zn, Co, and Ni (5 mM), and no precipitation occurred with elements such as Ca, K, and Mg tested at the same concentration as Cu (data not shown).

3.3. Experiment 3—In vivo Cu-sequestration by glomalin using two isolates of Glomus mosseae

No significant differences in Cu-sequestration by glomalin extracted from the two *Gl. mosseae* isolates were observed at the Cu levels tested (data not shown). The combined data for both isolates showed significant differences in Cu-sequestration by glomalin extracted from the hyphal ($P=0.05$) and root ($P=0.002$) compartments, but not from the sand compartment (Fig. 1). Glomalin from the hyphal compartments had higher Cu content than glomalin from sand in the hyphal compartment or from roots (Fig. 1). Additionally, the capacity for glomalin from hyphae to sequester Cu increased significantly at 10 μM Cu (1.60 mg Cu/g glomalin) and 20 μM Cu (1.63 mg Cu/g glomalin) in relation to no additional Cu (1.13 mg Cu/g glomalin).

González-Chávez et al. (2002) reported that after 20 min exposure in solution, AMF sequestered 3–14 mg Cu/g of dry hyphae. Glomalin represented 8% of the dry weight of the hyphae obtained from the hyphal compartment. This percentage was similar between fungi and Cu treatments. Therefore, using the results of González-Chávez et al. (2002), an empirical calculation of glomalin sequestration should be between 0.24 and 1.12 mg Cu/g glomalin. However, Cu values in glomalin from polluted soils were 1.5 to 4.29 mg Cu/g glomalin and 2 to 3 mg Cu/g for the in vivo experiment. It appears that glomalin has the potential to sequester Cu and is an important molecule to sequester this and other toxic elements. Arbuscular mycorrhizal fungi, thereby, may influence PTE availability at hyphosphere

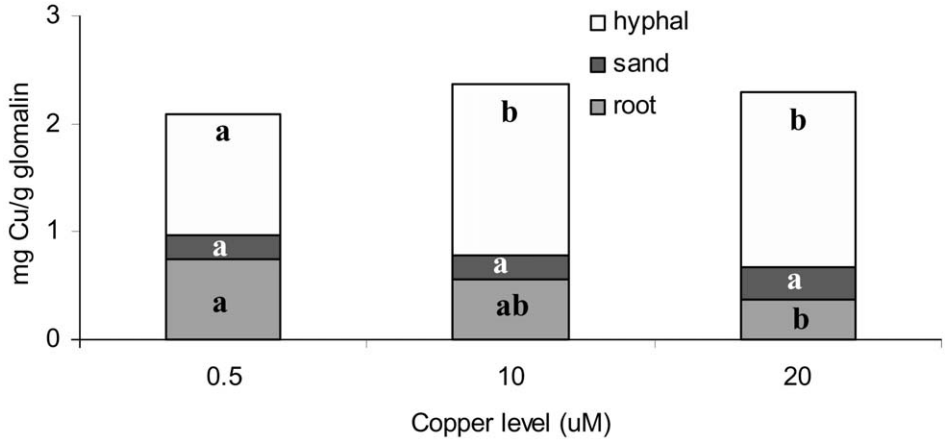

Fig. 1. Copper content of glomalin extracted from pot cultures of sorghum colonized by two isolates of *Glomus mosseae* (BEG-25 and BEG-132). A 38-μm mesh bag confined plant roots. Glomalin from the root zone and extraradical hyphae and sand from the root-free zone were analyzed separately. Means shown are for combined values of the two isolates (12 replicates). Different letters indicate significant differences for comparison made among sources of glomalin at each Cu level (Tukey's test, $P=0.05$). $LSD_{Cu\text{-}hyphal\ compartment}=0.44$, $LSD_{Cu\text{-}root\ compartment}=0.21$.

and soil rhizosphere levels. This also may be of significance for plant tolerance to PTEs.

Excised hyphae have the capacity to sequester Cu and Cd when exposed to solutions containing these cations (González-Chávez et al., 2002; Joner et al., 2000). González-Chávez et al. (2002) proved, however, that sequestration is a reversible mechanism. After a 5–20 min exposure to Cu, complete Cu-desorption was observed after acid extraction. It is possible that short exposure to Cu permits sorption mechanisms at the cell wall level, but after a longer time, accumulation rather than just sorption occurs. The in vivo experiment described herein was maintained for 9 weeks, but mycorrhizal plants were irrigated with increased Cu concentrations for just 6 weeks. A longer exposure to Cu may be necessary to show differences among AM fungal isolates in Cu sequestration. Also, the ability of an isolate to sequester high levels of a heavy metal may be lost during maintenance of the isolate by subculture. Isolate BEG-132 was from a polluted soil, but it had been maintained in sequential metal-free subcultures for 1–2 years before being used in our experiment. Recent work by Malcová et al. (2003) shows reduced Mn-tolerance of AM fungal isolates due to exclusion of stress levels of Mn from the growth medium used to maintain their fungal cultures.

González-Chávez et al. (2002) show that the hyphae of AMF sequesters Cu using transmission electron microscopy and scanning electron microscopy linked to an energy dispersive X-ray spectrometer. This occurs not only in the mucilaginous outer hyphal wall zone and the cell wall, but also inside the hyphal cytoplasm. The energy dispersive X-ray spectra also show that the accumulated Cu is mainly associated with Fe in the mucilaginous outer hyphal wall zone. However, the mechanisms involved were unclear. The results presented in the current study support the idea that hyphae of AMF play an important role in sequestration of PTEs. These results showed that glomalin may be the molecule involved in sequestration at the cell wall of hyphae, and because Fe is a major component of the glomalin molecule, Fe may be involved in the association with PTEs at the mucilaginous outer hyphal wall zone and the cell wall as observed by González-Chávez et al. (2002). For glomalin extracted from soils described in the current work, Fe concentration was high, especially in Soil 2 (44 mg Fe/g glomalin). The nature of PTE-binding by glomalin is unclear, however this protein may be one of the first cellular components in fungi coming into contact with the ions from the surrounding environment (Volesky, 1990).

4. Conclusion

Sequestration of PTEs by glomalin may be even higher than we reported because of desorption during processing of glomalin by the normal procedure for glomalin extraction and purification with citrate and hydrochloric acid. This glycoprotein may be stabilizing PTEs, reducing PTE availability and decreasing the toxicity risk to other soil microorganisms and plants growing in these sites. The copious production and the recalcitrance of this molecule in the soil (Rillig et al., 2001) further enhance the potential usefulness of these results. This study provides new information about the role of glomalin in the soil in addition to a role in soil aggregation (Wright and Upahadyaya, 1996) and as a major contributor to soil organic carbon (Nichols et al., 2002). These results indicated that glomalin participates in the sequestration of different PTEs. Glomalin sequesters Cu by reversible reactions and possibly to some extent by strong complexation. The glomalin pool in the soil may have a potential to sequestrate PTEs, not only by the colonized roots, but also by the hyphae and through deposition of glomalin in soil. Hyphal and glomalin production should be taken into account when phytostabilization technologies are used in polluted soils. This ability of AMF to sequester and accumulate PTEs in a non-toxic form may help to increase plant fitness and soil quality in polluted areas.

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References

Alloway, B.J., 1995. Heavy Metals in the Soil. Chapman and Hall, London.

Bradford, G.R., Page, A.L., Lund, J.L., Olmestead, W., 1975. Trace element concentrations of sewage treatment plants available lead, cadmium and molybdenum in mine tailings and contaminated soils. Agronomy Society of America, Madison, Wisconsin.

Bradley, R., Burt, A.J., Read, D.J., 1982. The biology of mycorrhiza in the Ericaceae. VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytologist 91, 197–201.

British Standard, 1995. Soil Quality Part 3. Chemical Methods. Section 3.9. Extraction of Trace Soluble Elements in Aqua Regia.

Cain, J.R., Allen, R.K., 1980. Use of a cell wall-less mutant strain to asses the role of the cell wall in cadmium and mercury tolerance by *Chlamydomonas reinhardtii*. Bulletin of Environmental Contamination and Toxicology 25, 797–803.

Chen, Y., Jurkevitche, E., Barness, E., Hadar, Y., 1994. Stability constants of Pseudobacterin complexes with transition metals. Soil Science Society of American Journal 58, 390–396.

Denny, H.J., Wilkins, D.A., 1987. Zinc tolerance in *Betula* spp. IV. Mechanisms of ectomycorrhizal amelioration of zinc toxicity. New Phytologist 106, 545–553.

Gadd, G.M., 1993. Interaction of fungi with toxic metals. New Phytologist 124, 25–60.

Gardea-Torresdey, J.L., Cano-Aguilera, I., Webb, R., Gutierrez-Corona, F., 1997. Enhanced copper adsorption and morphological alterations of cells of copper-stressed *Mucor rouxii*. Environmental Toxicology and Chemistry 16, 435–441.

González-Chávez, C., D'Haen, J., Vangronsveld, J., Dodd, J.C., 2002. Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant and Soil 240, 287–297.

Joner, E.J., Briones, R., Leyval, C., 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant and Soil 226, 227–234.

Kapoor, A., Virarghavan, T., 1995. Fungal biosorption—an alternative treatment option for heavy metal bearing wastewater: a review. Bioresource Technology 53, 195–206.

Lindsay, W.L., Norvel, W.A., 1978. Development of a DTPA test for zinc, iron, manganese and copper. Soil Science Society of American Journal 42, 421–428.

Macaskie, L.E., Dean, A.C.R., 1990. Metal-sequestering biochemicals. In: Volesky, B. (Ed.), Biosorption of Heavy Metals. CRC Press, Boca Raton, Florida, pp. 200–248.

Malcová, R., Rydová, J., Vosátka, M., 2003. Metal-free cultivation of Glomus sp.. BEG 140 isolated from Mn-contaminated soil reduces tolerance to Mn. Mycorrhiza 13, 151–157.

Millner, P.D., Kitt, D.G., 1992. The Beltsville method for soilless production of vesicular arbuscular mycorrhizal fungi. Mycorrhiza 2, 9–15.

Mittleman, M.W., Geesey, G.G., 1985. Copper-binding characteristics of exopolymers from a freshwater sediment bacterium. Applied and Environmental Microbiology 49, 846–849.

Morley, G.F., Gadd, G.M., 1995. Sorption of toxic metals by fungi and clay minerals. Mycological Research 99, 1429–1438.

Mullen, M.D., Wolf, D.C., Beveridge, T.J., Bailey, G.W., 1992. Sorption of heavy metals by the soil fungi *Aspergillus niger* and *Mucor rouxii*. Soil Biology and Biochemistry 24, 129–135.

Nichols, K., 2003. Characterization of Glomalin—A Glycoprotein Produced by Arbuscular Mycorrhizal Fungi. PhD Dissertation, University of Maryland, College Park, Maryland.

Nichols, K., Wright, S., Schmidt, W., Cavigelli, M., Dzantor, E., 2002. Carbon contribution and characteristics of humid acid, fulvic acid, particulate organic matter, and glomalin in diverse ecosystems. In: Proceedings of Humic Substances: Nature's most versatile materials, International Humic Substances Society, Boston, Massachusetts, pp. 365-367.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W., Tom, M.S., 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant and Soil 233, 167–177.

Ringbom, A., 1963. Complexation in Analytical Chemistry. Interscience Publishers, John Wiley and Sons, London.

Ross, S.M., 1994. Toxic Metals in Soil Plant Systems. John Wiley and Sons, London.

Spiro, T.G., 1981. Copper Proteins. John Wiley and Sons, New York.

Sterritt, R.M., Lester, J.N., 1986. Heavy metal immobilization by bacterial extracellular polymers. In: Eccles, H., Hunt, S. (Eds.), Immobilization of Ions by Bio-sorption, pp. 121–132.

Trappe, J.M., 1987. Phylogenetic and ecological aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir, G.R. (Ed.), Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Raton, Florida, pp. 5–25.

Volesky, B., 1990. Biosorption by fungal biomass. In: Volesky, B. (Ed.), Biosorption of Heavy Metals. CRC Press, Boca Raton, Florida, pp. 140–171.

Wright, S.F., 2000. A fluorescent antibody assay for hyphae and glomalin from arbuscular mycorrhizal fungi. Plant and Soil 226, 171–177.

Wright, S.F., Franke-Snyder, M., Morton, J.B., Upadhyaya, A., 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant and Soil 181, 193–203.

Wright, S.F., Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 161, 575–586.

Wright, S.F., Upadhyaya, A., 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil 198, 97–107.

Wright, S.F., Upadhayaya, A., Buyer, J.S., 1998. Comparison of N-linked oligosaccharides of glomalin form arbuscular mycorrhizal fungi and soils by capillary electrophoresis. Soil Biological and Biochemistry 30, 1853–1857.

Zhou, J.L., 1999. Zn biosorption by *Rhizopus arrhizus* and other fungi. Applied Microbiology and Biotechnology 51, 686–693.